THE PROTON SHIFT ACROSS THE MITOCHONDRIAL MEMBRANE ASSOCIATED WITH POTASSIUM UPTAKE INDUCED BY VALINOMYCIN

E.J.HARRIS

Department of Biophysics, University College, London, W.C.1. England

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1. Introduction

There are many respects in which the response of mitochondria to the addition of Ca^{2+} ions parallels that seen when a K^+ uptake is induced by addition of valinomycin in presence of an energy source. In each case a cation enters by an energy consuming process (Chance [1] for Ca^{2+}; Moore and Pressman [2] for K^+) and is accompanied to varying degrees by anions. The anion uptake is generally less than the equivalent of the cation gain because of permeability restrictions (Rossi, Scarpa and Azzone [3]; Harris [4]) and the electrical charge balance is preserved by movement of protons contrary to the Ca^{2+} or K^+. If the protons derive from a hitherto unionized group in the interior each proton will leave an anion equivalent behind to help balance the cation gain. This may explain observed stoichiometry of 1 H^+ ejected per Ca^{2+} gained (Rasmussen, Chance and Ogata [5]).

Mitchell and Moyle [6] demonstrated the production of external protons in response to the energy made available when a pulse of oxygen was applied to a previously anaerobic suspension in presence of EGTA to chelate Ca^{2+} and valinomycin (which increases the permeability to K^+). From this they argued that the proton production was more than a mere consequence of energised cation movement with its associated proton extrusion. They supposed that it represented the production of an energised state of the mitochondria. Hence it is relevant to present some observations made with a system which is already energised and in which responses are elicited by valinomycin addition (in presence of potassium ions). Such responses resemble those obtained by addition of Ca^{2+} ions to an energised system. In each case there is an apparent shift of internal pH in the alkaline direction and it is suggested that in all variants of the experiment the common basis is energised cation uptake.

2. Methods

Rat liver mitochondria were prepared using 0.25 M sucrose with 1 mM EDTA for homogenisation and 0.25 M sucrose for the three washes by resuspension and for the final suspension.

An indication of the local pH in some region of the mitochondria is obtainable by observation of the extinction of adsorbed bromthymol blue (BTB) (Chance and Mela [7]). The absorption measurements were made in a dual wavelength spectrophotometer excatly as described by these authors. In the cuvette there were also electrodes to monitor pH (Thomas combination type 4848-L 15) and K^+ concentration (Beckman 39047). The outputs from the electrodes were fed into electrometers and these in turn fed pen recorders. With the apparatus it was possible to record simultaneously the change in extinction at 618 nm with 700 nm as reference, and the pH and $[K^+]$ in the medium.

Addanki, Cahill and Sotos [8] have used the accumulation of the weak acid, dimethyl isoxazolidinedione(DMO) to deduce the internal pH of mitochondria. The application of this method requires knowledge of the appropriate water space in the particles and also of the total water associated with the particles since the part of the latter not in the matrix will carry DMO at the applied, external, concentration. Measurements were made in some ex-

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periments of the quantities of ^{14}C-labelled DMO and triated water carried by the mitochondria when they were centrifuged through a silicone layer exactly as described by Harris and van Dam [9]. In similar parallel experiments the quantities of ^{14}C-labelled sucrose and of tritiated water were determined. The combination of results gave the total DMO, the sucrose accessible water and the tritiated water space, the difference between the two latter gives the water inaccessible to sucrose which is assumed to act as solvent for the excess DMO over that carried in the sucrose accessible space.

The potassium carried down in the mitochondria was measured in portions of the acid extract (as described by Harris and van Dam [9]) using atomic absorption spectrometry.

3. Results

When valinomycin is added to mitochondria having an energy supply and in presence of adsorbed BTB there is a transient change in the absorption spectrum to that characteristic of the alkaline condition shown in fig. 1 by the change in the difference: O.D. at 618 nm–700 nm. The alkalinisation occurring during the time K^+ is being gained and H^+ is being lost. The direction of the BTB change then reverses and there is an increasing development of acidity during the period of K^+ retention, this time-dependent acidification is presumably due to metabolic activity stimulated by valinomycin. In the experiment illustrated the changes in the external pH and $[K^+]$ correspond to K^+ gain/H^+ loss = 147/62, each number being the μmoles/g protein. In similar conditions Harris and van Dam [9] showed that valinomycin causes malate to be gained but it is important that there was a lag between the K^+ uptake and that of the malate. From this there is reason to suppose that K^+ uptake proceeds so rapidly that proton ejection leaves an excess of hydroxyl ions, together with the anions of weakly acid internal groups, and that there is subsequently an exchange between hydroxyl and anions entering from the external medium. It is noteworthy that even the low ambient level of free BTB in the medium is measurably diminished when the K^+ is taken up and tends to increase when K^+ is lost in response to nigericin. That is to say, BTB acts like other penetrating

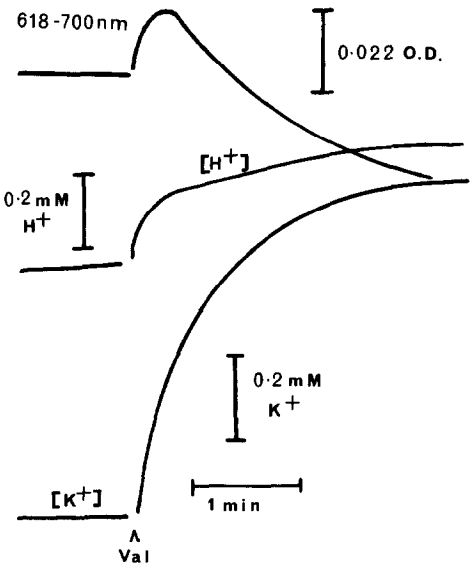

Fig. 1. Tracings of the recorded responses of a K^+ electrode, a pH electrode and the absorption due to bromthymol blue measured as the difference between signals at 618 and 700 nm when valinomycin (Val) is added at 62 μg/g protein to a suspension of rat-liver mitochondria (5.3 mg protein/ml). The medium contained sucrose 250 mM, KCl 5 mM, tris chloride 20 mM pH 7.4, tris glutamate and malate 3 mM each.

acids. In a particular case the free BTB in the medium fell from 0.64 μM to 0.22 μM when the mitochondrial K^+ increased in response to valinomycin and the external BTB returned to 0.40 μM when the K^+ fell after nigericin addition.

Turning now to the use of DMO as a way to deduce the internal pH using the formulation of Addanki et al. [8] the successive values obtained before, during K^+ uptake and at K^+ discharge are shown, along with the K^+ contents in fig. 2. When K^+ is gained DMO is also gained from the external solution in which it was at 1 mM initially. The increase in DMO is taken to be of the anion so that with the assumption of an even distribution of the un-ionised form and the figure for pK of Addanki et al. the internal pH is found to rise during the K^+ uptake. Since the DMO has been supposed to accumulate only in the water inaccessible to sucrose the present pH values exceed those of Addanki et al. [8], who regarded the total mitochondrial water as being the solvent. This difference does not affect the qualitative nature of the changes seen in response to valino-

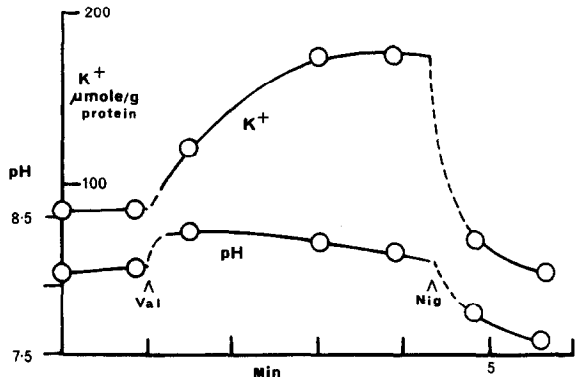

Fig. 2. Measurements of K^+ uptake and pH as deduced from DMO accumulation in the sucrose-inaccessible water of rat-liver mitochondria in response to an addition of valinomycin (Val, at 20 μg/g protein) and subsequently to nigericin (Nig at 60 μg/g protein). Medium as fig. 1.

mycin and nigericin; the K^+ uptake caused by the former agent is associated with an alkalinisation, probably greatest at the commencement of the process, and K^+ discharge is accompanied by acidification.

4. Discussion

Both methods used to estimate the internal pH show that qualitatively there is an alkalinisation at the time K^+ uptake is initiated by valinomycin addition. This change resembles that accompanying Ca^{2+} uptake (Chance and Mela [7]). In each case a cation is gained and protons are lost from the mitochondria. The pH gradient might occur because the interior was driven more positive when cations were being actively gained, as suggested by Harris and Pressman [10]. In the experiments described and unlike those of Mitchell and Moyle [6] the starting condition was energised and the mitochondria would have phosphorylated ADP added with phosphate. It seems then that the development of the pH gradient in the conditions of figs. 1 and 2 depends on initiating an energy consuming cation uptake. It seems correct to expect that if the K^+ uptake is set off by adding oxygen to a de-energised system (as used by Mitchell and Moyle [6]) with valinomycin to provide the requisite high permeability to K^+ then there will be an appearance of protons and a transient pH gradient.

Using data published by Harris, Cockrell and Pressman [11] the estimated H^+ output/atoms of O respired ratio would be between 2.5 and 5 with glutamate and malate as substrate mixture. With β-hydroxybutyrate as substrate a higher ratio would be expected because this anion does not accompany K^+, in contrast to malate (Harris [4]). Accordingly the observation of protons pulses in response to oxygen in presence of valinomycin and K^+ described by Mitchell and Moyle seem to be part of the set of changes which active cation uptake engenders rather than symptomatic of membrane energisation *per se*. Recent observations (Thomas, Manger and Harris [12]) show that proton pulses obtained in the response to oxygen when no valinomycin has been added depend on there being a sufficient level of free Ca^{2+} in the medium so again they reflect an accompaniment of active cation uptake.

Acknowledgements

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